UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Transmittal of the Meeting Minutes of the Endocrine Disruptor Methods Validation Subcommittee under the National Advisory Council for Environmental Policy and Technology (NACEPT), held June 11, 2002.

TO: Dorothy Bowers, Chair
National Advisory Council for Environmental Policy and Technology
Office of Cooperation and Environmental Management

And

Gwen Whitt, Designated Federal Official
National Advisory Council for Environmental Policy and Technology
Office of Cooperation and Environmental Management

FROM: Jane Scott Smith, Designated Federal Official
NACEPT Endocrine Disruptor Methods Validation Subcommittee
Office of Science Coordination and Policy/OPPTS

THRU: William Benson, PhD., Director
Gulf Ecology Division, NHEERL, Office of Research and Development
Co-Chair of Endocrine Disruptor Methods Validation Subcommittee

Please find attached the minutes of the NACEPT Endocrine Disruptor Methods Validation Subcommittee fourth open meeting and first teleconference held in Washington, D.C. June 11, 2002. This meeting summary covers the Detailed Review Paper (DRP), for Steroidogenesis.

Information about NACEPT EDMVS meetings and activities can be obtained from the website at http://www.epa.gov/scipoly/oscpendo or the OPPT Docket, OPPT 2002-0020 at (202) 566-0280. Interested persons are invited to contact Jane Smith, EDMVS Designated Federal Official (DFO), via e-mail at smith.jane-scott@epa.gov.

cc:

Sonia Altieri
Charles Auer
Dennis Deziel
Susan Hazen
Stephen Johnson
James Jones
Joseph Merenda
Marcia Mulkey
Gordon Schisler
Margaret Schneider
Adam Sharp
OPPT Docket 2002-0020

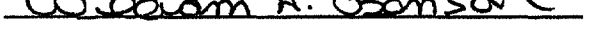
MEETING MINUTES
OF
ENDOCRINE DISRUPTOR METHODS VALIDATION SUBCOMMITTEE
A Subcommittee of The National Advisory Council for Environmental Policy and Technology

TELECONFERENCE ON
JUNE 11, 2002
AT
RESOLVE, 1255 23RD STREET, N.W. SUITE 275
WASHINGTON, D.C.

This meeting summary covers the Presentation and discussion of the Steroidogenesis Detailed Review Paper (DRP).

Jane Scott Smith, DFO
Endocrine Disruptor Methods Validation Subcommittee under the National Advisory Council for Environmental Policy and Technology

Date: 9/18/2002



William Benson, PhD., CO-Chair
Endocrine Disruptor Methods Validation Subcommittee under the National Advisory Council for Environmental Policy and Technology

Date: 09.18.02

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EDMVS Members in Attendance at the March 2002 Teleconference

Attended in Person at RESOLVE

William Benson, Ph.D., Vice Chair
U.S. EPA

Theodora Colborn, Ph.D.
World Wildlife Fund

Attended Via Telephone

Mildred Christian, Ph.D.
Argus Research

Robert Combs
Director, FRAME

Rodger D. Curren, Ph.D.
Institute for In Vitro Sciences, Inc

Peter L. deFur, Ph.D.
Virginia Commonwealth University

J. Charles Eldridge, Ph,D.
Wake Forest University

Robert J. Kavlock, Ph.D.
U.S. EPA

William Kelce, Ph.D.
Pharmacia Corporation

Timothy Kubiak, M.P.H.
U.S. Fish and Wildlife Service

Gerald A. LeBlanc, Ph.D.
North Carolina State University

Ron Miller, Ph.D
The Dow Chemical Company

Susan C. Nagel, Ph.D.
University of Missouti-Columbia

James W. "Willie" Owens, Ph.D.
The Procter & Gamble Company

Thomas L. Potter, Ph.D.
USDA-Agriculture Research Service

Theodore H. Schettler M.D., MPH
Science & Environ. Health Network

James T. Stevens. Ph.D.
Syngenta

William Stokes, D.V. M
NIEHS

Glen Van Der Kraak, Ph.D.
University of Guelph

Facilitator
Paul De Morgan
RESOLVE

Designated Federal Official
Jane Scott Smith
Office of Science Policy and Coordination

Presenter

Presenter

Jerry Goldman
EPA, ORD

Oral Public Commenter

Oral Statement

Troy Seidle
People for the Ethical Treatment of Animals

NOTICE

This meeting summary has been written as part of the activities of the National Advisory Council on Environmental Policy and Technology (NACEPT), Endocrine Disruptor Methods Validation Subcommittee (EDMVS). This meeting summary has not been reviewed for approval by the United States Environmental Protection Agency (Agency) and, hence, the contents of the meeting summary do not necessarily represent the views and policies of the Agency, nor of other agencies in the Executive Branch of the Federal government, nor does mention of trade names or commercial products constitute a recommendation for use.

The NACEPT EDMVS was established in partial fulfillment of a Congressional statute. When Congress amended the Federal Food Drug and Cosmetics Act (FFDCA) in the Food Quality Protection Act (FQPA) of 1996, it directed the U.S. Environmental Protection Agency (EPA) to develop a screening program to determine whether certain substances may have hormonal effects in humans. To ensure that EPA has the best and most up-to-date advice available regarding the validation of the screens and tests in the EDSP, EPA established the Endocrine Disruptor Methods Validation Subcommittee (EDMVS) under the NACEPT. The EDMVS provides independent advice and counsel to the Agency through NACEPT on scientific and technical issues related to validation of the EDSP Tier I and Tier II assays, including advice on methods for reducing animal use, refining procedures involving animals to make them less stressful, and replacing animals where scientifically appropriate. The EDMVS held their first meeting in October of 2001, their second meeting in December 2001, and their third meeting in March 2002. The fourth meeting of the EDMVS was conducted as an international teleconference in June 2002.

The June 11, 2002 open meeting (teleconference) of the EDMVS was announced in the Federal Register on May 28, 2002 (Volume 67, Number 102). Further information about NACEPT EDMVS meetings and activities can be obtained from its website at http://www.epa.gov/scipoly/oscpendo or the OPPT Docket at (202) 260-7099. INTERESTED PERSONS ARE INVITED TO CONTACT Jane Smith, EDMVS Designated Federal Official (DFO), via e-mail at smith.jane-scott@epa.gov.

National Advisory Council for Environmental Policy and Technology (NACEPT)
Endocrine Disruptor Methods Validation Subcommittee (EDMVS)

Meeting by Conference Call
June 11, 2002
10:00 AM – 12:00 noon EDT
DRAFT Agenda

Members of the public may join this conference call in person at the conference room in the RESOLVE offices at 1255 23rd St. NW, Suite 275, Washington, DC. To register to participate by phone, please contact Jane Smith, designated federal official for the EDMVS, at smith.jane-scott@epa.gov or 202/564-8476.

Meeting Objective:

- Provide comments and advice on the Steroidogenesis DRP (Tier I).

10:00 – 10:05 Phoning in

10:05 - 10:10 Roll Call

10:10 – 10:40 Steroidogenesis DRP (Tier I)
Jerry Goldman, NHEERL, ORD, EPA

10:40 – 11:40 Discussion on Steroidogenesis DRP

Discussion questions:

1. Does the EDMVS agree with the recommendation of the DRP that EPA should commence prevalidation studies on the sectioned testis assay for steroidogenesis?
2. If yes to #1: Approximately 250 mg or 1/4 of an adult SD rat testis is the sample size generally described by investigators. Should the EPA conduct a study to investigate the sensitivity of the preparations of testicular tissue less the 250 mg to determine an optimal and/or threshold amount to use?
3. The DRP recommends that the test substances listed below be evaluated during prevalidation. Does the EDMVS agree with the choice of test substances?
   - bisphenol A (inhibits steroidogenic signal transduction)
   - lindane (inhibits signal transduction and the StAR protein)
   - ketoconazole (a weak imidazole anti-fungal; inhibits $P450_{scc}$ and aromatase)
   - genistein (a weak phytoestrogen/flavanoid; inhibits 3β-HSD)
   - flutamide (inhibits P450c17)
   - econazole (a potent imidazole; inhibits aromatase)
   - aminoglutethimide (positive control; inhibits $P450_{scc}$)
   - finasteride (negative control; inhibits 5α-hydroxylase)
4. Should EPA investigate the use of MA-10, R2C and H295R cell lines as alternatives to the sectioned testis assay in the EDSP? If so, which cell line?

11:40 – 11:55 Public Comment

Members of the public will be given an opportunity to comment and are requested to focus their comments on issues related to the Steroidogenesis DRP to the extent possible. The amount of time given to each individual will depend on the number of people wishing to provide comment.

11:55 – 12:00 Next Steps and Agenda for July 23-24, 2002 Meeting

12:00 Adjourn

Introduction

The Office of Science Policy and Coordination's Endocrine Disruptor Screening program established the Endocrine Disruptor Methods Validation Subcommittee (EDMVA) under The National Advisory Council for Environmental Policy and Technology (NACEPT). The first EDMVS meeting was held in October 2001. That initial meeting brought the members together to review the mission statement and discuss subcommittee roles and responsibilities. The second meeting, held in December 2001, was the first time the subcommittee members were presented with specific questions regarding assay protocols. The third meeting, held March 2002, continued discussions on protocols as well as some discussions on the validation process, Core Chemicals, 'low dose' and means of assessing human health effects. This fourth meeting, held as a teleconference, was wholly concerned with the Steroidogenesis assay.

Endocrine Disruptor Methods Validation Subcommittee (EDMVS)
Meeting by Conference Call
June 11, 2002

Draft Meeting Summary

On June 11, 2002, the U.S. Environmental Protection Agency (EPA) convened a meeting of the EDMVS by conference call. The objective of the meeting was to provide comments and advice on the *Draft Detailed Review Paper on Steroidogenesis Screening Assays and Endocrine Disruptors*. The meeting took place in Washington, DC; however, many of the EDMVS members, as well as some members of the public, participated by phone.

Copies of presentation slides and other materials distributed at the meeting may be obtained by contacting Jane Smith, the designated federal official for EDMVS, at smith.jane-scott@epa.gov or 202/564-8476. The materials also are available on the EPA website at http://www.epa.gov/scipoly/oscpendo/edmvs.htm. EPA has established an administrative record for this meeting under docket control number 2002-0020. The docket is available for inspection in the TSCA Non-confidential Information Center, 1200 Constitution Ave., Washington, DC. The center is open from noon to 4:00 p.m., Monday through Friday, excluding legal holidays. The center's phone number is (202) 566-0280.

I. Opening Comments, Roll Call, and Agenda Review

Paul De Morgan, senior mediator with RESOLVE, welcomed EDMVS members and other participants to the meeting and thanked them for attending by phone or in person. He noted that there was some disappointment that the full three-day EDMVS meeting was postponed until July, but observed that the reason for the delay was to ensure the products being reviewed by the EDMVS were adequately prepared. He explained that because the Steroidogenesis Detailed Review Paper (DRP) was ready for EDMVS comment, the conference call was scheduled to provide EPA with input in as timely a manner as possible. Ms. Smith indicated EPA was interested in determining whether the conference call approach, on an as needed basis, could be

utilized by the EDMVS in the future and thanked the members for trying the approach.

Mr. De Morgan did a roll call of EDMVS members and asked other participants to announce their names. A list of the meeting attendees is attached (see Attachment A). He noted that the time constraints and logistics of meeting by conference call might limit the ability to discuss the issues as fully as some would like. He encouraged participants to submit written comments to Ms. Smith if they were unable to raise them on the call. He then reviewed the meeting agenda.

II. Steroidogenesis DRP

Gary Timm, EPA Office of Science Coordination and Policy (OSCP), began the presentation by indicating the purpose of the DRP was to survey the literature and identify the types of studies needed for validation. He referred EDMVS members to the questions listed in the agenda, noting these were specific areas where EPA would like EDMVS comments. He then introduced Jerry Goldman, National Health and Environmental Effects Research Laboratory (NHEERL), Office of Research and Development (ORD), EPA, to present an overview of the DRP.

Dr. Goldman began by noting that the DRP is very extensive. He outlined the actions of the hypothalamic-pituitary-gonadal axis and the pathways of sex steroid synthesis. He then listed the factors for consideration in selecting a screening approach to evaluate a toxic effect on steroidogenesis, commenting that no one approach would rank at the top for all the factors:

- Predictiveness
- Sensitivity
- Variability
- Animal use
- Ease of use
- Standardization
- Cost
- Time requirements
- Multiple samples evaluated
- Metabolic activation

Dr. Goldman explained that the choice of protocols involves four issues: gender, type of exposure, biological material, and sampling. He compared the strengths and limitations of *in vitro* and *in vivo* approaches. The DRP includes a review of four *in vitro* approaches: isolated gonadal organs, sectioned/minced tissue, primary cell preparations, and cell lines. Dr. Goldman reviewed the strengths and limitations of the sliced testis method, which is the approach recommended in the DRP. He noted that the DRP did not include consideration of the recent paper by Powlin et al. (Tox. Sci. 46:61, 1998), but indicated the Powlin paper would not affect the conclusions in the DRP. He also outlined the strengths and weaknesses of the isolated cell preparation approach and reviewed the primary considerations in the selection of cell lines.

Dr. Goldman explained that the DRP recommendation was to use a quartered testis approach with *in vitro* exposure. The DRP includes a second recommendation to explore the feasibility of using a cell line as an alternative. While an assessment of cytoxicity is commonly employed in cell culture work, such evaluations for tissue maintained *in vitro* are less straightforward, but

essential. A number of possible approaches were mentioned, including lactic dehydrogenase leakage, ATP measures using a bioluminescence assay, and determinations of cytokine release (if the compound under study does not typically trigger a cytokine response apart from an effect on cell toxicity). In addition to the chemicals recommended in the DRP, Dr. Goldman listed six other possible candidates for prevalidation: ketoconazole, cyanoketone, trilostane, dimethoate, aminoglutethimide, and prochloraz.

Following the presentation a member noted that the need for additional expertise to maintain cell cultures should not be listed as a limitation of the isolated cell approach if the cells do not require anything beyond normal cell culture techniques.

III. EDMVS Member Discussion of the Steroidogenesis DRP

Mr. De Morgan referred members to the discussion questions listed in the agenda and asked for their input on the first question:

1. *Does the EDMVS agree with the recommendation of the DRP that EPA should commence prevalidation studies on the sectioned testis assay for steroidogenesis?*

A number of members commented that a lot of work and science went into the DRP, and they agreed with much of the analysis. However, they also indicated it did not present a sufficient case to support the recommendation that EPA go ahead with the sectioned testis approach. Rather, they indicated, it suggests that EPA should proceed with studies on both the sectioned testis and the cell line approaches. One member noted the DRP indicates that the sectioned testis approach is less sensitive than purified cell preparations and commented that sensitivity will be important if the assay is used for prioritization.

Members also suggested further side-by-side comparison of the two approaches. One member noted that it is likely that EPA's Office of Research and Development will pursue further studies on the feasibility of using cell lines as an alternative to minced tissue. Another member suggested that examining available cell line data and doing a comparison on performance would help EPA make an objective decision.

One member commented that the first step should be to establish a method for cytotoxicity, which might help to clarify the choice of approach. A member responded that it is unlikely a test for cytotoxicity in minced testis can be developed. Another member commented that focusing on cytotoxicity runs the risk of setting up a situation in which a compound is ignored if it is cytotoxic even though it may affect testosterone synthesis in a different way. One member noted that several easy assays exist to test cytotoxicity.

A member commented that EPA should be clear about the potential use of the steroidogenesis assay before choosing and prevalidating an approach. Mr. Timm responded that the assay is a candidate for the tier 1 battery, being recommended by the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC), adding that the battery will not be chosen until the results from all the candidate assays can be compared. As a follow-on comment, the member noted that although some have pushed for inclusion of the Intact Male Screening assay, the

current candidates for the tier 1 battery do not include another assay that could serve as a double check for steroidogenesis. Mr. Timm noted that a compound would not be moved to tier 2 testing based on the results of a single *in vitro* tier 1 assay. A member added that efforts must proceed under the premise that the steroidogenesis assay, like any included in the tier 1 battery, will contribute to the weight of the evidence. The pubertal male may obviate the need for the steroidogenesis assay. It is believed that the pubteral female would not. The proponents of the adult male assay believe that it would detect steroidogenesis inhibitors but with any of these there is the question of sensitivity compared with the *in vitro* assay.

One member commented that he was surprised that the Powlin et al. paper was omitted from the DRP as the paper is one of the few that attempted a side-by-side comparison within assays. DRP author Dr. Jerry Johnson, Battelle, apologized for the omission and explained that it had been an accidental oversight due to the key word search parameters used. Dr. Goldman commented that the data presented in the paper indicated to the authors that a sliced testis approach using *in vitro* toxicant exposures correctly predicted effects for only four (17β-estradiol, ketoconozole, flutamide, and haloperidol) of nine tested chemicals. They believed that the results for anastrozole, aminoglutethimide, finasteride, reserpine, and the estrogen receptor antagonist ICI-182,780 were not predictive. Dr. Goldman said, however, that he had some reservations about the authors' conclusions, specifically:

(1) The Powlin et al. paper stated that the results with the aromatase inhibitor aminoglutethimide were not predictive because no change in estradiol secretion from the testis explants was present. They did note a decrease in the ovarian explants, but the very low level of estradiol production in the testes may have accounted for a lack of effect. Anastrozole was also deemed not predictive, but again testicular levels of aromatase are low, as the authors acknowledged.

(2) *In vitro* exposure to the highest concentration of the 5α-reductase inhibitor finasteride caused a dose-related decrease in testosterone and estradiol from the testis explants, something which the authors did not predict. However, the alterations may have been due to a cytotoxic response, since the high dose equaled the limit of solubility.

(3) Although it was not predicted, ICI-182,780 elevated estradiol at the highest dose in both the *in vitro* male and female explants, in addition to the *ex vivo* ovarian explants. There was sufficient consistency in the results that the authors offered a mechanistic explanation for the effect, suggesting that they believed the effect to be real. The prediction, not the assay, may have been at fault.

(4) Finally, the conclusions for haloperidol and reserpine were based upon spectral cytochrome P450 binding data from liver microsomes obtained from phenobarbital-treated rats. Summary data were not shown, and a question arises about the applicability of liver microsomal data to any testis effects, given that there are signficant differences in the cytochrome P450 distribution in tissue from the two organs and that haloperidol and reserpine have shown binding affinities to only some P450 enzymes.

Dr. Goldman noted one additional factor concerning the use of 50 versus 250 milligram (mg) explants. The Powlin paper used 50 mg parenchymal fragments although some data have indicated increased variability using the smaller sized fragments.

Members also requested further comparison of techniques within the sectioned testis approach; a specific suggestion was made for a table comparing the different techniques. One member commented that the DRP presents an assay that can go forward but more effort is needed to optimize the assay before moving into the formal prevalidation stage. Other members agreed and noted several parameters that should be optimized, including tissue stability, alternate sources, tissue section size, and incubation time.

Dr. Bill Benson, acting chair of the EDMVS, summarized the comments he had heard, including: EPA should explore both the sectioned testis and the cell line approaches; test optimization is an important stage and is especially needed for the sectioned testis assay, realizing that cytotoxicity is important; and more side-by-side data are needed on cell lines.

2. *Should EPA conduct a study to investigate an optimal and/or threshold amount of testicular tissue to use?*

Several members commented that it would be useful to optimize tissue sample size and generate data on variability before setting the protocol. One member suggested that rather than specifying a size the protocol should include a performance standard to allow for possible animal variability.

3. *Does the EDMVS agree with the choice of test substances?*

A member suggested that EPA focus on chemicals that get into the environment rather than pharmaceuticals. Another member commented that some papers have shown 3-methyl-SO2-DDE (3-methylsulfonyl-2,2-bis(4-chlorophenyl)-1,1-dichloroethyene) to be an effective positive control with the H295R cell line. A member suggested including a general cytotoxin.

One member asked whether all of the chemicals proposed for this assay would be run through the *in vivo* assays also for comparison. Mr. Timm commented that the question of a core set of chemicals for all assays would be addressed at the July EDMVS meeting.

4. *Should EPA investigate the use of MA-10, R2C and H295R cell lines as alternatives to the sectioned testis assay in the EDSP? If so, which cell line?*

Building on earlier conversations suggesting EPA explore the cell line approach, a number of members suggested moving ahead with the H295R cell line as it is well tested and available. Another member suggested pursuing a cell line that will also look at aromatase and estradiol.

IV. Public Comment

Troy Seidle, People for the Ethical Treatment of Animals

Mr. Seidle commented that the DRP provides an excellent overview of information on steroidogenesis. He voiced agreement, however, with several EDMVS members that the DRP did not make an overwhelming case for the sectioned testis approach. He requested that EPA pursue further efforts with cell lines, especially H295R. He also asked for EPA's position regarding the lack of metabolism in *in vitro* assays.

Mr. Timm responded that EPA has been exploring *in vivo* methods to get at metabolism. He said that the EDSTAC and others felt that both *in vivo* and *in vitro* methods were needed in tier 1. He noted that other options may be explored in the future, but currently, *in vivo* methods seem to be the best approach for capturing metabolism.

The meeting adjourned at 12:00 noon EDT.

Reflections and Next Steps

Sherry Sterling, acting director of EPA's OSCP, said that EPA is working to fill the EDMVS co-chair vacancy that resulted from the change in Dr. Vanessa Vu's position at EPA. She said that various people are under consideration, and EPA hopes to be able to announce the new co-chair at the July EDMVS meeting.

In addition, the following next steps were noted at the end of the meeting.

- Any additional comments on the steroidogenesis DRP should be submitted to Jane Smith.
- RESOLVE will draft a summary of this teleconference meeting and circulate it to EPA and EDMVS members for review and comment.
- EPA will notify the EDMVS as to the EPA's decisions or general direction on the various issues raised in the discussion questions (it was noted that EPA will be sharing their decisions regarding all questions asked of the EDMVS in the future).
- Within the next few weeks RESOLVE will distribute logistical information and a draft agenda for the July EDMVS meeting.